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Intestinal absorption and cell transforming potential of PhIP-M1, a bacterial metabolite of the heterocyclic aromatic amine 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)

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HIGHLIGHTS

- The transport of PhIP-M1, a metabolite of the colon carcinogen PhIP produced by gut bacteria, in eight different intestinal segments from male Fischer 344 rats was examined in Ussing chambers.
- A very recently developed HPLC method to quantify the concentration of PhIP-M1 in biological fluids as well as in intestinal tissue was applied.
- At the most, 10–20% of the PhIP-M1 amount added to the mucosal compartment of the Ussing chambers per segment were absorbed within 90 min.
- PhIP-M1 in a concentration of up to 100 μM did not induce the malignant transformation of BALB/c 3T3 cells.
- Even if one would assume that 100% of the daily amount of PhIP ingested by a human being is converted into PhIP-M1 in the colon, this concentration most probably would not lead to carcinogenicity in the colorectal mucosa.

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ABSTRACT

Previous studies have shown that in the rat, the colon carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is only absorbed to a limited extent in the small intestines and that a major fraction of unmetabolised PhIP reaches the colon. Moreover, PhIP is extensively metabolised when incubated with human stool samples to a major derivative, 7-hydroxy-5-methyl-3-phenyl-6,7,8,9-tetrahydropyrido[3′,2′:4,5]imidazo[1,2-*a*]pyrimidin-5-ium chloride (PhIP-M1). In the present study, the uptake and transport of PhIP-M1 in Ussing chamber experiments, its cytotoxicity in the different segments of the Fischer 344 rat gut and its transforming potential in the BALB/c 3T3 cell transformation assay were analysed. At the most, 10–20% of the PhIP-M1 amount added to the mucosal compartment of the Ussing chambers per segment were absorbed within 90 min. Therefore, the amount of PhIP-M1 detected in the tissues as well as in the serosal compartment of the Ussing chambers was extremely low. Moreover, human-relevant concentrations of PhIP-M1 were not cytotoxic and did not induce the malignant transformation of BALB/c 3T3 cells. In conclusion, even if one would assume that 100% of the daily amount of PhIP ingested by a human being is converted into PhIP-M1 in the colon, this concentration most probably would not lead to cytotoxicity and/or carcinogenicity in the colorectal mucosa.

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1. Introduction

Epidemiological studies in the past indicate that a correlation between the consumption of high amounts of strongly heated red meat and the development of colorectal cancer may in fact exist (Scheppach et al., 1999; Chao et al., 2005; Norat et al., 2005; Sinha et al., 2005; Wu et al., 2006; Rohrmann et al., 2007). As to the compounds possibly being involved in the malignant transformation of epithelial cells in the colon and rectum, a number of toxic substances, including the heterocyclic aromatic amines (HCAs), have been identified in heated red meat. Among the different HCAs identified, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the most abundant and has therefore been postulated to play an important role in colon cancer development (Layton et al., 1995). In this context, it has been shown that PhIP is mutagenic (Thompson et al., 1987), binds to DNA (Dragsted et al., 1995), forms DNA adducts (Frandsen et al., 1992; Schut and Herzog, 1992; Friesen et al., 1994) and leads to the formation of tumors in the large intestine of the rat (Ito et al., 1991; Hasegawa et al., 1993).

In order to induce tumor formation in the large intestines of the rat, the feed used in the studies by Ito et al. (1991) and Hasegawa et al. (1993) included very high concentrations of PhIP (100–400 mg/kg feed). If the final concentration of PhIP in the lab chow was below 50 mg/kg feed, no significant increase in the number of preneoplastic and neoplastic lesions was observed in the colon of rats when compared to control animals (Fukushima et al., 2004; Doi et al., 2005; Kühnel et al., 2009). These results actually suggested that PhIP is only absorbed to a limited extent in the rat intestine, and, in fact, Nicken et al. (2010) demonstrated that less than 10% of PhIP added to the mucosal compartment of Ussing chambers was absorbed by the rat small intestines. In this context it should be mentioned that when rats were administered [^{14}C] PhIP by gavage 50% of the dose appeared in the faeces as unchanged PhIP (Watkins et al., 1991).

If one takes into account that PhIP is a colon carcinogen in the rat and that a major fraction of unmetabolised PhIP reaches the rat colon, the possibility that bacteria in the colon metabolise PhIP to a toxic metabolite has to be considered. Vanhaecke et al. (2006) were indeed able to show that PhIP is extensively metabolised when incubated with human stool samples to a major derivative, 7-hydroxy-5-methyl-3-phenyl-6,7,8,9-tetrahydropyrido[3′,2′:4,5] imidazo[1,2-*a*]pyrimidin-5-ium chloride (PhIP-M1). Moreover, PhIP-M1 was identified in urinary and fecal samples of human volunteers having eaten cooked chicken containing PhIP (Vanhaecke et al., 2008a). The metabolisation of PhIP to PhIP-M1 is catalysed, among others, by strains of the species *Enteroccocus faecium*,*Enteroccocus durans, Enteroccocus avium* and *Lactobacillus reuteri*, and the anaerobic fermentation of glycerol via 3-hydroxypropionaldehyde is an essential step in the formation of PhIP-M1 (Vanhaecke et al., 2008b). Regarding the toxicity of PhIP-M1, Vanhaecke et al. (2008c) showed that 100–200 μM PhIP-M1 leads to a concentration-dependent increase in DNA damage in Caco-2 cells when tested in the alkaline comet assay.

In the present study, the uptake and transport of PhIP-M1, its cytotoxicity in the different segments of the Fischer 344 rat gut and its transforming potential in the BALB/c 3T3 cell transformation assay were analysed. Furthermore, the relevance of PhIP-M1 in colon carcinogenesis is discussed with regard to previously published results and those obtained in the present study.

2. Materials and methods

2.1. Chemicals

PhIP-M1 (97% purity) was obtained through incubation of PhIP with human fecal samples followed by liquid/liquid extraction and preparative HPLC purification as described in Vanhaecke et al. (2006). A 10 mM stock solution of PhIP-M1 in 0.01% formic acid was prepared for the Ussing chamber experiments, the final concentration of PhIP-M1 in the mucosal compartment of the Ussing chambers at the beginning of the experiments being 10 μM. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine-d3 (PhIP-D3) was purchased from Toronto Research Chemicals (North York, Canada).

2.2. Animals

Male Fischer 344 rats were obtained from Charles River Laboratories International Inc. (Sulzfeld, Germany) and housed for a minimum of 8 days in our animal facility. During this period of time, the animals had free access to water and standard feed. Rats were sacrificed at an age of 8–10 weeks (body weight: 200 ± 40 g). The study protocol was approved by the Animal Welfare Service of the Lower Saxony State Office for Consumer Protection and Food Safety (Oldenburg, Germany).

2.3. Preparation of the intestinal segments

Rats were killed by cervical dislocation and subsequent exsanguination. The whole gut was removed from the abdomen within the first 5 min after sacrificing the animals and kept in ice-cold buffer used for the serosal compartment (see Section 2.4), which was continuously gassed with carbogen (95% O_2: 5% CO_2), until the tissues were stripped and mounted into the Ussing chambers. The following gut segments were used: the duodenal sample was taken 1 cm distal to the pylorus, the proximal jejunal sample 15 cm distal to the duodenum, the distal jejunal sample 10 cm proximal to the ileum, the ileal sample directly proximal to the caecum and the caecal sample from the *corpus ceci*. The tissue excised up to 3 cm distal to the junction of the caecum and the colon was defined as “proximal colon”, the tissue proximal to the rectum as “distal colon” and the tissue immediately proximal to the anus as “rectum”.

2.4. Ussing chamber experiments

From each animal and location two about 2 cm long segments were used. The epithelial tissues were stripped from the muscle and the serosal layers on ice and mounted into the Ussing chambers (Ussing and Zerahn, 1951) with an exposed serosal area of 0.5 cm^2 as previously described in detail (Breves et al., 2000;

Table 1
Composition of the buffers in the mucosal and serosal compartments of the Ussing chambers used for the study of small and large intestinal samples[a].

Constituents	Small intestine		Large intestine	
	Mucosal	Serosal	Mucosal	Serosal
NaCl	113.6[b]	113.6	53.6	113.6
KCl	5.4	5.4	5.4	5.4
HCl (1.0 N)	0.2	0.2	0.2	0.2
$MgCl_2 \times 6H_2O$	1.2	1.2	1.2	1.2
$CaCl_2 \times 2H_2O$	1.2	1.2	1.2	1.2
$NaHCO_3$	21	21	21	21
$Na_2HPO_4 \times 2H_2O$	1.5	1.5	1.5	1.5
Glucose	–	10	–	10
Mannitol	2	2	2	2
HEPES	20	7	10	7
NaOH (1.0 N)	6	–	–	–
Godium gluconate	–	6	6	–
Sodium acetate × $3H_2O$	–	–	36	–
Sodium propionate	–	–	15	–
Sodium butyrate	–	–	9	–
Indomethacin	0.01	0.01	0.01	0.01

[a] Osmolality of the buffers: 300 mOsm/kg (at pH 7.4–7.5 and 37 °C after gassing withcarbogen).
[b] The concentration of the buffer constituents is expressed in mmol/l.